Expression of integrins v 3 and v 5 and their ligands in primary and secondary central nervous system neoplasms

Mittelbronn, M ; Warth, A ; Meyermann, R ; Goodman, S ; Weller, M

Abstract: Aims: To study the expression of integrins v 3 and v 5 and their ligands in tumour, stroma and endothelial cells from human glioblastoma and CNS metastases from breast, lung and skin tumours. Methods and results: Integrin and integrin ligand expression was quantified in frozen tumour surgical specimens (15 glioblastomas and breast carcinoma metastases as well as 16 lung carcinoma and melanoma metastases) using immunohistochemistry. Gene expression profiles were evaluated in glioblastomas (n=424) and in normal brain (n=11). Overall, v 3 expression was more common than v 5 except in tumours derived from lung. v 3 expression was most frequent in glioblastomas and melanoma metastases. Most lung-derived tumours expressed v 5 but expression was less frequent in other tumours; about 20% of breast-derived tumours strongly expressed v 5. Melanoma-derived tumours did not express v 5. Expression of integrin ligands vitronectin, fibrinogen, fibronectin and osteopontin was variable between tumours, although most tumours expressed the ligands to some extent. Marked v 3, but not v 5, expression was common in stroma of CNS metastases. In blood vessels, v 3 expression was more frequent than v 5 and more pronounced in CNS metastases than in glioblastomas. Integrin ligand expression occurred in blood vessels in most tumours. In glioblastomas, mRNA expression of v 3, v 5, osteopontin and fibronectin were significantly upregulated over normal brain. Conclusions: Overall, we report distinct and heterogeneous patterns of integrin expression in primary and secondary brain tumours that may be relevant to the future development of integrin-targeting therapeutic approaches to brain tumours.

Expression of integrins αvβ3 and αvβ5 and their ligands in primary and secondary central nervous system neoplasms

Running title: Integrin expression in CNS neoplasms

Key words: cancer, integrin expression, tumour microenvironment

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Abstract

Aims: To study the expression of integrins αvβ3 and αvβ5, and their ligands in tumour, stroma and endothelial cells from human glioblastoma and CNS metastases from breast, lung and skin tumours.

Methods and results: Integrin and integrin ligand expression was quantified in frozen tumour surgical specimens (each 15 glioblastomas and breast carcinoma metastases as well as each 16 lung carcinoma and melanoma metastases) using immunohistochemistry. Gene expression profiles were evaluated in glioblastomas (n=424) and in normal brain (n=11). Overall, αvβ3 expression was more common than αvβ5, except in tumours derived from lung. αvβ3 expression was most frequent in glioblastomas and melanoma metastases. Most lung-derived tumours expressed αvβ5, but expression was less frequent in other tumours; about 20% of breast-derived tumours strongly expressed αvβ5. Melanoma-derived tumours did not express αvβ5. Expression of integrin ligands vitronectin, fibrinogen, fibronectin and osteopontin was variable between tumours, although most tumours expressed the ligands to some extent. Marked αvβ3, but not αvβ5, expression was common in stroma of CNS metastases. In blood vessels, αvβ3 expression was more frequent than αvβ5 and more pronounced in CNS metastases than in glioblastomas. Integrin ligand expression occurred in blood vessels in most tumours. In glioblastomas, mRNA expression of αvβ3, αvβ5, osteopontin and fibronectin were significantly upregulated over normal brain.

Conclusions: Overall, we report disinct and heterogenous patterns of integrin expression in primary and secondary brain tumours that may be relevant to the future development of integrin-targeting therapeutic approaches to brain tumours.

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Introduction

Integrins are important in promoting tumour development and metastasis. The interaction of integrins with the extracellular matrix influences the assembly and organisation of the intracellular cytoskeleton, enhances cell survival and reduces the likelihood of apoptosis in a variety of tumour types (Janes and Watt, 2006; Le Tourneau et al., 2007; Moschos et al., 2007; Streuli, 2009). The centres of growing solid tumours can become hypoxic, which prompts the generation of new blood supply for the tumour through angiogenesis, in which integrins expressed by the growing endothelia influence migration to and entry into the tumour (Nikolopoulos et al., 2004; Varner and Cheresh, 1996).

Activation of integrins αvβ3 and αvβ5 has been implicated in these cellular processes (Enns et al., 2005; Hodivala-Dike, 2008; Beauvais et al., 2009; Somanath et al., 2009). In mice with tumours derived from intracranially injected glioblastoma cells, the tumours had lower vessel density in αvβ3-deficient (knockout) than in wild-type mice (Kanamori et al., 2006). Similarly, inhibition of integrins, including αvβ3, inhibited tumour growth in a nude rat model (Mikkelsen et al., 2009). Conversely, increased activation of αvβ3 increased the ability of cultured cells to migrate (Verbisck et al., 2009). A further experimental study showed that selective inhibition of αvβ3 and αvβ5 integrins with an Arg-Gly-Asp (RGD) peptide induced endothelial cell death via anoikis (cell death associated specifically with detachment from the extracellular matrix) (Maubant et al., 2006). In human glioblastoma, the expression of αvβ3 appears to be higher in higher-grade tumours (glioblastomas World Health Organization [WHO] grade IV relative to low-grade astrocytomas), but integrin inhibition induces only detachment, but not anoikis (Schnell et al., 2008; Maurer et al., 2009). As the growth of a tumour is influenced

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strongly by its environment (Kanamori et al., 2006), mechanisms related to the expression of αvβ3 and αvβ5 are likely to be relevant to both primary central nervous system (CNS) tumours and metastases from other tumour types. Here we have studied the expression profile of αvβ3 and αvβ5 and their principal ligands (vitronectin, fibrinogen, fibronectin, osteopontin) in primary human CNS neoplasms (WHO grade IV glioblastomas) and secondary neoplasms in the brain originating from primary tumours in the lung, breast and skin (melanoma) to elucidate potential differences in the expression profile of primary and secondary malignant CNS neoplasms. The expression pattern of these proteins was studied in tumour parenchyma, stromal cells, and in blood vessels. Gene expression profiles of the integrins and their ligands derived from open-source databases were also evaluated in brain tumours and compared with their expression in normal CNS tissues.

Materials and Methods

mRNA expression of integrins and integrin ligands in primary tumour tissues and normal tissues

Differences in the gene expression profile in primary glioblastomas (n=424) versus normal CNS tissues (n=11) were assessed from two publicly available platforms at the US National Institute of Health: The Cancer Genome Atlas (TCGA) Data Portal (http://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp) and the REpository for Molecular BRAin Neoplasia DaTa (REMBRANDT) (https://caintegrator.nci.nih.gov/rembrandt/). Both platforms contain clinical information, genomic characterisation data, and high-throughput sequencing analysis of tumour genomes and provide researchers with the ability to perform ad hoc querying across multiple data domains. Since for secondary brain tumours (breast and lung) only *z*-scores, but no expression ratios (mRNA expression *vs* normal tissue), were available, these data were not included in the present study.

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Preparation of tumour samples and immunohistochemical staining

Tumour tissue samples were immediately frozen in liquid nitrogen ((each 15 glioblastomas and breast carcinoma metastases as well as each 16 lung carcinoma and melanoma metastases)). Tumour diagnoses were confirmed by at least two neuropathologists. Sections of 10 µm thickness were cut using a microtome and mounted on Super Frost Plus slides (Microm International, Walldorf, Germany). Sections were dried, and postfixed for 10 min in ethanol at 4°C, followed by acetone for 1 min and Tris-buffered saline for 5 min at room temperature. For automatic immunohistochemical tissue labelling, the Benchmark immunohistochemistry system (Ventana, Strasbourg,

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France) was used. Endogenous peroxidase of the tissue sections was blocked with 3% H_2O_2 in methanol for 14 min. A cell-conditioning pretreatment was performed. Primary antibodies (Table 1) were applied at a working concentration of 10 µg ml^{-1} for 30 min. An avidin and a biotin blocker were applied to the samples for 4 min, respectively, followed by an 8-min incubation with one drop of I-View-Biotin Ig (Ventana). For diaminobenzidine (DAB) visualisation, the sections were incubated with one drop of I-View streptavidin–horseradish peroxidase for 8 min and then with DAB/H_2O_2 for an additional 8 min. Sections were finally incubated with a copper enhancer (Ventana) for 4 min, then washed, counterstained with haematoxylin and mounted. Negative and isotype controls were conducted for all tumours.

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Quantification of expression in tissue sections

For all tumours, only sections where staining could be unequivocally attributed to blood vessels, tumour cells, or stromal cells were evaluated. In CNS metastases, stromal cells were defined as non-neoplastic tissue intermingled between tumor cell clusters, blood vessels and/or CNS tissue. Astrocytic tumours do not develop stroma, and consist of diffusely infiltrating tumour cells which invade preexisting CNS parenchyma, thus, expression on stromal cells could not be assessed in these tumours. M. Mittelbronn and D. Capper quantified staining intensity and frequency separately for blood vessels, tumour and stromal cells (see above), using an Olympus BX50 microscope. Staining frequency for tumour and stromal cells was categorised as: 0, no staining; 1, single cells positive (focal pattern); 2, single cells positive (diffuse pattern); 3, up to 20% positive cells; 4, 20–50% positive cells; or 5, >50% positive cells. Staining frequency for blood vessels was categorised as: 0, all vessels negative; 1, moderate number of positive

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blood vessels; or 2, most blood vessels positive. Staining intensity was categorised as: 1, weak; 2, moderate; or 3, strong. Statistical analysis was performed by a contingency table analysis using χ^2 tests.

Results

Expression of αvβ3 and αvβ5 integrins in tumor cells of primary and secondary CNS tumours

Figure 1 summarises the expression of αvβ3 and αvβ5 integrins in tumor cells of primary and secondary CNS tumours and Figure 2 shows representative immunohistochemical stainings. αvβ3 was more commonly expressed than αvβ5 in tumour cells, except for lung carcinomas, in which the tumor cells showed a slightly higher expression frequency for αvβ5 than for αvβ3. Glioblastomas and melanoma-derived brain metastases were most likely to express αvβ3 in a moderate or high proportion of tumor cells (20-50% or >50%). Although, αvβ3 expression was detected in most tumour samples, about one-third of lung- and breast-derived brain metastases had no αvβ3 expression. The intensity of staining in all samples expressing αvβ3 was generally weak (score 1, data not shown). In contrast, six out of seven lung-derived brain tumours showed αvβ5-expressing tumor cells. Expression of this integrin was less frequent in the other tumour types: there was no expression of αvβ5 in melanoma-derived and most glioblastomas or breast-derived brain metastases did also not express αvβ5. Nevertheless, approximately 20% of breast-derived brain tumours strongly expressed αvβ5. In general, in glioblastomas, integrin αvβ3 was much more strongly expressed as compared to αvβ5. Expression intensity of αvβ3 was generally weak in secondary brain tumours, with only one sample scoring higher than 1 (a single breast carcinoma which scored 2 [moderate]).

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Expression of integrin ligands in tumor cells of primary and secondary CNS tumours

The frequency of expression of integrin ligands is summarised in Figure 3 and representative immunohistochemical stainings are shown in Figure 4. About half of the glioblastomas and breast-derived brain metastases expressed fibrinogen, although the frequency of expression was mostly in <50% of cells. Stronger expression of fibrinogen was observed in tumor cells derived from melanoma or especially lung carcinoma metastases. Staining intensities for fibronectin in tumor cells were mostly weak, with moderate staining intensity observed only in a single glioblastoma sample and in two lung-derived tumours. Between one-third and two-thirds of samples from each tumour type did not express fibronectin, and no primary or secondary CNS neoplasm expressed fibronectin in >50% of all tumor cells. Vitronectin was moderately or strongly expressed in all secondary brain tumours studied. In contrast, in glioblastoma, most samples did not show a reproducible staining result so that these stainings were not included in the evaluation. Osteopontin was expressed by tumor cells of most glioblastomas and the majority of lung- and melanoma-derived tumours, whereas expression of this ligand was uncommon in breast-derived brain tumours. Intensity scores for osteopontin in glioblastoma cells were mostly moderate (9/14), with four samples demonstrating weak and one sample strong intensity.

Expression of αvβ3 and αvβ5 integrins and their ligands in stromal cells of secondary CNS neoplasms

Intrinsic brain malignancies do not usually develop stromal elements, so analysis of stromal expression was restricted to metastatic brain tumours (Table 3). Marked

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Gelöscht: Between one-third and two-thirds of samples from each tumour type did not express fibronectin, and no tumour expressed fibronectin in >50% of cells.

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Gelöscht: About half of the glioblastoma and breast-derived brain metastases expressed fibrinogen, although the frequency of expression was mostly in <50% of cells (Fig. 3). Stronger expression of fibrinogen was observed in melanoma-derived and especially lung-derived brain metastases. Staining intensities for fibronectin were mostly weak, with moderate staining intensity observed only in a single glioblastoma sample and in two lung-derived tumours.

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Gelöscht: Expression intensity for osteopontin in breast- and lung-derived brain tumours was roughly evenly divided between weak and moderate intensity, with a single tumour in each case demonstrating strong intensity. The expression intensity was strongest for osteopontin in melanoma-derived tumours, where nine tumours demonstrated moderate, one tumour strong, and only one tumour weak intensity. Only weak staining intensity was observed for vitronectin, in the few samples available.

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expression of αvβ3 was observed in tumor-associated stromal cells of the majority of lung- and melanoma-derived tumours, and in 40% of breast tumours. αvβ5 expression was uncommon in breast tumour stroma and was not studied in lung- or melanoma-derived tumours since the stromal cells were not unequivocally assessable for αvβ5 in these samples. The majority of the metastatic brain tumours showed marked stromal expression of fibrinogen, vitronectin and fibronectin (Table 3). In contrast, strong expression of osteopontin was only seen in single cases.

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Expression of αvβ3 and αvβ5 integrins and their ligands on blood vessels of primary and secondary CNS neoplasms

Most tissue specimens of breast-, lung- and melanoma derived tumours showed expression of αvβ3 in most tumor-associated blood vessels, whereas only a minority of glioblastoma-associated vessels expressed αvβ3. αvβ5 in blood vessels was generally absent or only moderately frequent in both primary and secondary CNS neoplasms. Most tumours expressed the integrin ligands in blood vessels at moderate or high frequency (Table 4).

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mRNA expression of integrins and integrin ligands in glioblastomas compared to normal CNS tissues

Figure 5 illustrates integrin and integrin ligand mRNA expression in glioblastomas relative to the normal CNS tissues. cDNA expression of αvβ3 and αvβ5 was significantly upregulated in glioblastomas (n = 424) as compared to normal brain (n = 11) (P = 0.0003 and P = 0.0072, respectively). Regarding the expression of integrin ligands, osteopontin demonstrated the strongest upregulation compared with normal brain tissue

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(P < 0.0001). Fibronectin was also significantly upregulated (P < 0.0001), whereas vitronectin and fibrinogen expression in glioblastomas did not significantly differ from those in normal brain tissue.

Discussion

We have studied the expression of αv integrins and their ligands in primary and metastatic tumours in human brain specimens. Our primary findings are that integrin αvβ3 was expressed by most glioblastomas and in brain metastases from breast and lung cancers and melanomas, often at high frequencies. Integrin αvβ5 was expressed at low frequency in all tumours except in those from lung, where expression frequency was higher than that for αvβ3. Our data from glioblastoma samples show that glioblastomas express αvβ3 and αvβ5 integrins, both on neoplastic astrocytes and on invasive blood vessels (Gladson and Cheresh, 1991; Paulus et al., 1993; Gladson, 1996; Bello et al., 2001; Kanamori et al., 2004). In contrast to the constantly high αvβ3 expression on blood vessels in CNS metastases, αvβ3 expression was considerably lower in primary CNS glioblastomas. In contrast to CNS metastases, glioma vascularisation is provided in a cooptive manner for a long time (Fischer et al., 2005). In CNS metastases, activated blood vessels have to invade actively the cohesive tumor cels clusters. Glioma cells often migrate along preexisting blood vessels thereby gaining access to oxygen and nutrients. This might be at least partly responsible for a later and more moderate activation of glioma-associated blood vessels in contrast to a generally stronger expression of integrins in blood vessels associated with CNS metastases. Potentially, αvβ3 is just turned on in a distinct temporo-regional manner in case of insufficient supply by vascular cooption. In general, our data on integrin expression in secondary brain tumours are consistent with reports of αvβ3 and αvβ5 expression in primary breast cancer (Meyer et al., 1998; Silvestri et al., 2002; Havaki et al., 2007; Vellon et al., 2007; Beer et al., 2008), lung cancer (Chen et al., 2005; Cui et al., 2007; Fong et al., 2009), and melanoma (Neto et al., 2007; Tzukert et al,. 2010), where the expression of αvβ3

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was suggested to be linked to the invasive potential of the tumour cells. Our study also defines the expression patterns of αvβ3 and αvβ5 in tumour stroma and endothelial cells within the different tumour types. Results in breast- and lung-derived brain metastases are consistent with earlier reports of αvβ3 expression in capillaries of primary breast and lung carcinomas (Max et al., 1997).

Expression of integrin ligands was variable in tumour tissues. For all secondary brain tumours, vitronectin was the most commonly expressed integrin ligand evaluated. The abundant expression of this ligand has been previously reported in primary breast cancer (Aaboe et al., 2003); however, to our knowledge, not in the other tumours we describe here. The strong expression of vitronectin is all the more of interest since emerging evidence especially presents the vitronectin/αvβ3 axis as an important pathway affecting intracellular signalling in cancer progression (Reuning, 2011). αvβ3 was found in more than 50% of all tumor entities at least to some extent in our cohort while αvβ3 was absent in all melanomas and approximately 70% of breast carcinomas. These findings suggest that especially the inhibition of the vitronectin/αvβ3 pathway could be a promising target for secondary brain tumors. Concerning vitronectin expression in glioblastomas, other studies showed that most human astrocytic tumors are completely negative for vitronectin. Interestingly, only „loose focal vitronectin deposits“ were described in single cases of glioblastoma in one study (Zámecník et al., 2003). This is quite in line with our findings, however we did not feel comfortable with the staining results and were not convinced that such „loose focal vitronectin deposits“ really represent specific staining signals. Furthermore, also the fact that vitronectin seems slightly downregulated in glioblastomas as compared to normal CNS tissue (see figure 5) might be an additional indicator for our interpretation that the vitronectin staining

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signals in glioblastomas are not reliable. In general, about half of the tumours expressed the other ligands, with a low frequency of expression of fibronectin and osteopontin in breast-derived metastases, and a higher expression of fibrinogen in lung-derived brain metastases. While a high expression of fibrinogen has been demonstrated in primary lung cancer tissue (Sierko et al., 2012), our data regarding low osteopontin expression in breast-derived brain metastases diverge from its reported high expression in primary breast carcinoma (Kim et al., 1998). The expression of integrin ligands did not clearly follow expression of individual integrins, despite vitronectin being described as a monospecific ligand for αvβ5, while fibrinogen and osteopontin preferentially target αvβ3 (Hynes, 2002). Osteopontin has been described as increasing the migratory potential of cancer cells via activation of αvβ3 (Fong et al., 2009). The majority of tumours expressed osteopontin. The high levels of osteopontin expression in glioblastomas could be experimentally linked to an increased glioma cell invasion and proliferation (Jan et al., 2010), correlate with the extent of angiogenesis *in vivo* (Matusan-Ilijas et al., 2008) and could meanwhile be established as a serum biomarker for worse patient prognosis in human gliomas (Sreekanthreddy et al., 2010). Concerning the expression of integrins and their corresponding integrin ligands in primary tumors or cells a similar heterogeneity similar to our study was described (Marshall et al., 1991; Taherian et al., 2011).

Overall, some tumours demonstrated high-frequency expression of integrins and their ligands. In addition, in glioblastomas, cDNA expression of αvβ3, αvβ5, osteopontin and fibronectin were significantly upregulated compared with normal CNS tissues. The role of αvβ3 and αvβ5 integrins in tumour development and metastasis is being exploited clinically. Cilengitide, a cyclised Arg-Gly-Asp (RGD)-containing peptide (Cyclo-

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[Asp-D-Phe-N-MeVal-Arg-Gly]), is a selective inhibitor of these integrins, and is currently being studied in the clinics in various tumours settings, including glioblastoma (Reardon et al., 2008; Stupp et al., 2010). Further studies are needed to reveal whether the expression of αvβ3 *vs* αvβ5 we have measured in brain tumours is related to hypoxia or ischaemia which can upregulate αvβ3 (but not αvβ5) (del Zoppo et al., 2006), or if this expression profile is a phenomenon intrinsic to the tumour itself.

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Conclusions

Integrins αvβ3 and αvβ5 in human glioblastoma and in CNS metastases from lung and breast cancer, and melanoma are expressed iin a distinct and heterogenous expression pattern. The differential expression of integrins and integrin ligands may help to develop targeted integrin-antagonistic therapies for human brain cancers.

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Michel Mittelbronn 4.7.12 08:22
Gelöscht: Lopes M.B. (2003) Angiogenesis in brain tumors. Microsc. Res. Tech. 60, 225-230.

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Michel Mittelbronn 4.7.12 08:33
Gelöscht:

Michel Mittelbronn 15.7.12 14:00
Gelöscht: Rangaswami H., and Kundu G.C. (2007) Osteopontin stimulates melanoma growth and lung metastasis through NIK/MEKK1-dependent MMP-9 activation pathways. Oncol. Rep. 18, 909-915.

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Michel Mittelbronn 15.7.12 14:18
Formatiert: Einzug: Links: 1,27 cm, Hängend: 0,63 cm

Michel Mittelbronn 15.7.12 14:18
Gelöscht:

Michel Mittelbronn 15.7.12 14:19
Formatiert: Einzug: Links: 2 cm

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Michel Mittelbronn 15.7.12 14:59
Gelöscht:

Table 1. Antibodies[a], clones, and providers.

Michel Mittelbronn 15.7.12 12:57
Gelöscht:

Antibody	**Clone**	**Provider**
Fibrinogen	Polyclonal	Dako Cytomation, Carpinteria, CA, USA
Fibronectin	Polyclonal	Dako Cytomation, Carpinteria, CA, USA
αvβ3	Lm609 (IgG1)	Millipore, Schwalbach, Germany
Osteopontin	Polyclonal	NeoMarkers, Lab Vision Corporation, CA, USA
αvβ5	P1F6 (IgG1)	Millipore, Schwalbach, Germany
Vitronectin	153	Dr D. Seiffert, Scripps, La Jolla, CA, USA

[a]Rabbit polyclonal antibodies were used. IgG1, immunoglobulin 1.

.

Table 2. Numbers of frozen samples analysed (glioblastoma and central nervous system metastases of different origin).

Antibody:	**αvβ3**	**αvβ5**	**Fibrinogen**	**Fibronectin**	**Osteopontin**	**Vitronectin**
Glioblastoma	15	10	15	15	14	1
Breast	15	10	10	10	9	14
Lung	16	7	11	10	11	15
Melanoma	16	9	9	11	11	14

Table 3. Data on expression of integrins and integrin ligands in stromal cells: numbers of tumours demonstrating minor/marked expression.

Tumour origin[a]:	**Breast**	**Lung**	**Melanoma**
αvβ3	9/6	3/6	2/4
αvβ5	4/1	–[b]	–[b]
Vitronectin	2/8	0/10	0/6
Fibronectin	2/6	0/7	0/3
Osteopontin	6/1	5/1	1/1
Fibrinogen	1/7	0/9[c]	1/3

Each table entry shows the number of tumours with minor expression (expression frequency scores 0–3)/numbers of tumours with marked expression (expression frequency scores 4–5). χ^2 analysis indicated no significant differences within the dataset. [a]Tumours of central nervous system origin do not display stromal cells and this analysis was therefore not conducted for glioblastoma samples. [b]Insufficient number of samples for analysis. [c]All samples had frequency score 5 (expression in >50% of cells).

Table 4. Data on expression of integrins and integrin ligands in tumor-associated blood vessels.

Tumour origin:	Glioblastoma	Breast	Lung	Melanoma
αvβ3	9/4/2	4/1/9	1/3/12	5/5/6
αvβ5	5/4/0	6/2/2	3/4/0	3/6/0
Vitronectin	–[a]	2/1/10	0/1/12	0/3/11
Fibronectin	0/0/15	0/2/8	1/1/8	3/1/7
Osteopontin	0/4/10	0/4/5	1/3/4	1/3/4
Fibrinogen	3/3/8	0/5/5	0/3/8	1/4/5

Data shown refer to sections with blood vessel frequency score = 0 (no expression in blood vessels [lowest category])/blood vessel frequency score = 1 (moderate number of positive blood vessels/blood vessel frequency score = 3 (expression in most blood vessels). [a]Insufficient number of samples for analysis.

Michel Mittelbronn 5.7.12 17:58
Gelöscht: capillaries

Michel Mittelbronn 5.7.12 17:58
Gelöscht: capillary

Michel Mittelbronn 5.7.12 17:58
Gelöscht: capillaries

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Michel Mittelbronn 5.7.12 17:58
Gelöscht: capillary

Michel Mittelbronn 5.7.12 17:58
Gelöscht: capillaries

Figure legends

Figure 1. Expression of αvβ3 and αvβ5 integrins in tumor cells of glioblastomas and central nervous system metastases from breast and lung cancer or melanoma.

Michel Mittelbronn 5.7.12 11:58
Gelöscht: Expression of αvβ3 and αvβ5 integrins in glioblastomas and central nervous system metastases from breast and lung cancer or melanoma.

Figure 2. Immunhistochemical analysis of αvβ3 (a-d) and αvβ5 (e-f) integrins in glioblastomas (a, b and e, f) and central nervous system metastases from breast (c) and lung (g, h) cancer or melanoma (d) (original magnification 200x for all images; scale bar = 100µm).

Michel Mittelbronn 5.7.12 12:03
Gelöscht: Expression of αvβ3 in (a) glioblastoma and (b) and lung-derived brain carcinoma samples.

Figure 3. Expression of integrin ligands in tumor cells of glioblastomas and central nervous system metastases from breast and lung cancer or melanoma. FBG, fibrinogen; FTN, fibronectin; OPN, osteopontin; VTN, vitronectin. Vitronectin expression was not analysed in glioblastomas as only one sample was available.

Figure 4. Immunhistochemical analysis of fibrinogen (a, b), fibronectin (c, d), osteopontin (e, f) and vitronectin (g, h) in glioblastomas (a, c, e) and central nervous system metastases from breast (d, h) and lung (b, f) cancer or melanoma (g) (original magnification 200x for all images; scale bar = 100µm).

Michel Mittelbronn 5.7.12 12:05
Gelöscht: Expression of intregrin ligands. (a) vitronectin expression in lung-derived brain carcinoma and (b) osteopontin expression in glioblastoma.

Figure 5. mRNA expression of integrins and their ligands in glioblastomas (n=424) relative to normal central nervous system tissues (n=11). The data derives from The Cancer Genome Atlas (TCGA) Data Portal (https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp). For this study, the TCGA Portal has been assessed in November 2011. Log2-fold mRNA expression changes are depicted.

Michel Mittelbronn 5.7.12 12:14
Gelöscht: cDNA

Michel Mittelbronn 5.7.12 12:11
Gelöscht: